6. SUMMARY OF IMMUNOGENICITY

6.1. Introduction

Immunogenicity endpoints have been assessed in more than 1,400 children and more than 400 adults.

An overview summary of the primary results and conclusions from these studies in children and adults is presented in Table 28.

Table 28
(1 of 2)
Summary of Primary Immunogenicity Conclusions from Studies of FluMist in Children or Adults

Population	Study Phase	Objectives	Primary Results	Conclusions
Children	Study AV002 and AV002-2 Phase 1 and 2	Identify safe and immunogenic dose of FluMist compared to placebo when administered by spray or drops in healthy children 18–71 months of age.	The serum HAI antibody response following a single dose of FluMist administered by nasal spray was similar to that induced by nasal drops. Immunogenicity of FluMist increased in a dose-dependent manner; $10^5 - 10^7$ $TCID_{50}$, induced serum HAI antibody responses in children.	Intranasal spray delivery of $10^6 - 10^7$ $TCID_{50}$ dosage of FluMist is safe and immunogenic in children.
	Study AV006 Phase 3 Year One	Assess safety, immunogenicity, and efficacy of one or two doses of FluMist compared to placebo in healthy children 15–71 months of age.	Both one- and two-dose regimens were highly immunogenic and efficacious against H3N2 and B strains. A two-dose primary regimen of FluMist was more immunogenic in children for the Type A/H1N1 strain than a single-dose primary regimen.	A one-dose or two-dose primary vaccination regimen is immunogenic and protects children against the Type A/H3N2 and Type B strains.
	Year Two	Assess safety, immunogenicity, and efficacy of a single re-vaccination dose of FluMist compared to placebo in healthy children.	Following re-vaccination, an increase in antibody to H1N1 and B strains was observed, and antibody to H3N2 remained high; the cumulative seropositivity rate was 82%, 100%, and 99% to H1N1, H3N2, and B strains, respectively. Efficacy against homologous H3N2 and B strains was 100%. Efficacy against the drifted strain A/Sydney/05/97 (H3N2) was 86%.	Regardless of pre-vaccination immune status, there was an immune response to annual re-vaccination. A high serum HAI antibody response rate has high positive predictive value for efficacy of FluMist in children. FluMist induced a heterotypic protective immune response.

Table 28
(2 of 2)
Summary of Primary Immunogenicity Conclusions from Studies of FluMist in Children or Adults

Population	Study Phase	Objectives	Primary Results	Conclusions
Children	Study AV011 Phase 3	Assess immunogenicity and efficacy of a single re-vaccination dose of FluMist against challenge with monovalent H1N1 vaccine strain, compared to placebo in healthy children.	Re-vaccination of children with a single dose of FluMist induced protective immunity against challenge with the H1N1 vaccine strain five to eight months later. Efficacy against H1N1 challenge was 83%. Protection correlated with the presence of systemic and nasal antibodies, but protection was observed even in the absence of either serum or nasal antibodies.	Trivalent FluMist protects against H1N1. Vaccination with FluMist induces multiple immunological mechanisms. There is no single correlate of immunity for FluMist in children.
	Study AV007 Phase 3	Demonstrate similar safety, tolerability and immunogenicity of two doses of each of three consistency lots of FluMist.	An immune response was consistently induced following two doses of FluMist, regardless of which consistency lot was administered.	Clinical consistency was demonstrated.
	Study AV014 Phase 3	Demonstrate similar safety, tolerability and immunogenicity of two doses of FluMist blended and filled at Medeva compared to FluMist blended and filled at Aviron-PA.	FluMist induced a comparable immune response, regardless of the facility in which it was blended and filled.	Clinical consistency was demonstrated.
Adults	Study AV003 Phase 3 Challenge Study	Assess immunogenicity and efficacy of FluMist or licensed inactivated influenza vaccine compared to placebo.	Efficacy of FluMist was 85% against all strains combined, and efficacy of TIV against all strains combined was 71%. The serum HAI antibody response induced by TIV was higher than that induced by FluMist.	FluMist protects healthy adults against influenza. Vaccination with FluMist induces multiple immunological mechanisms. Low serum HAI antibody responses do not have negative predictive value for FluMist efficacy.

6.1.1. Immunological Assays

The four immunological assays used in FluMist studies are summarized in Table 29. The serum HAI assay was used as the primary assay because it measures the immune response to the viral HA antigen in a specific, rapid, simple, accurate, and reproducible manner. Furthermore, HAI antibodies are functional in that they block attachment of the viral HA to sialic acid-containing receptors present on the cell surface. Blocking of viral attachment to erythrocytes is the mechanism whereby HA-specific antibodies inhibit hemagglutination, and blocking of viral attachment to epithelial cells is one mechanism whereby HA-specific antibodies neutralize influenza virus infectivity. Therefore, the HAI assay was a surrogate assay for virus neutralizing antibodies. ELISA and micro-neutralization were also used as supplementary immunogenicity assays in some studies.

Table 29
Immunological Assays and Types of Antibodies Detected

Assay	Specimens Tested	Antigen Used in Assay	Major Specificity of Antibody Detected in Assay	Function of Antibody Detected in Assay
HAI	Serum	Whole influenza virions	HA	Inhibition of viral hemagglutination and, by analogy, inhibition of virus attachment to host cells
IgG ELISA	Serum	Detergent-disrupted influenza virions (enriched to contain predominantly HA)	HA	Binding to viral hemagglutinin (IgG antibodies)
Micro-neutralization	Serum	Whole infectious influenza virions	HA (NA antibodies also can participate)	Inhibition of virus attachment and entry into cells and/or release from infected cells
IgA ELISA	Nasal Wash	Detergent-disrupted influenza virions (enriched to contain predominantly HA)	HA	Binding to viral hemagglutinin (IgA antibodies)

6.2. Immunogenicity of FluMist in Adults

6.2.1. Study Designs and Demographics

Aviron has completed five studies with immunogenicity endpoints in adults. These five studies have enrolled 444 adults who were evaluated for their immune response to vaccination with FluMist. Four unique trivalent vaccine formulations were used (Table 30).

Table 30
FluMist Studies in Adults with Immunogenicity Endpoints

Study	Number Enrolled	Vaccine Strain Formulation	Dose Regimen
AV001	239	A/Texas/36/91 (H1N1) A/Shangdong/9/93 (H3N2) B/Panama/45/90	One
AV003	103	A/Texas/36/91 (H1N1) A/Shangdong/9/93 (H3N2) B/Panama/45/90	One
AV004	20	A/Texas/36/91 (H1N1) A/Johannesburg/33/94 (H3N2) B/Panama/45/90	One
AV005	32	A/Texas/36/91 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	Two
AR001	40 [a]	A/Shenzhen/227/95 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	One

[a] Of the 384 adults enrolled in Study AR001, 40 were enrolled in the immunogenicity substudy.

Immunogenicity studies of FluMist in adults were conducted in a population that was primarily White (78%) and reasonably balanced with respect to gender (44% male). The mean age of FluMist recipients was 34 years.

6.2.2. FluMist Adult Dosage and Regimen

The safety and immunogenicity of the 10^7 $TCID_{50}$ dosage was demonstrated in five clinical studies in adults with FluMist.

The rationale for the one-dose vaccination regimen for adults was supported by Aviron trials and previously published studies that showed that one dose of CAIV was safe, immunogenic, and efficacious in adults against natural or investigational challenge with wild-type H1N1, H3N2, or B influenza strains. Use of a one-dose regimen in adults was further supported by epidemiological data, which indicates that individuals older than six years of age are likely to have serum antibodies to the major influenza subtypes that are responsible for disease in humans. Since most adults, therefore, have been immunologically primed, they should require only a single dose of vaccine to stimulate an immune response to FluMist.

Study AV003 demonstrated that a single dose of FluMist protected adults against challenge with wild-type influenza, and Study AV009 demonstrated that a single dose of FluMist reduced the occurrence of severe febrile illness and febrile upper respiratory illness during the influenza season and the illness-associated missed work and healthcare utilization.

In summary, the immunogenicity endpoints in FluMist studies, the efficacy study of FluMist in adults (Study AV003), the effectiveness study of FluMist in adults (Study AV009), previous efficacy studies with CAIV, and epidemiological data showing age-related acquisition of immunity to influenza all support use of a single-dose regimen of FluMist in adults.

6.2.3. Relationship Between Immunogenicity and Efficacy in Adults

Immunogenicity and efficacy of FluMist in adults were evaluated in Study AV003, which consisted of a vaccination phase and a challenge phase. Participants were randomized to receive FluMist, TIV, or placebo. A serum sample was taken from all participants prior to vaccination and four weeks after vaccination. Each participant was challenged with a wild-type virus that was antigenically equivalent to one of the three constituent vaccine strains and to which he or she had been serosusceptible prior to vaccination. Serum was also obtained from each of the 92 participants prior to and 28 days following the challenge with wild-type influenza for serum HAI testing. An immune response was defined as a ≥4-fold increase in serum HAI titer from baseline to Day 28 following vaccination or from Day 28 to Day 56 following challenge.

The serum HAI antibody response rate of serosusceptible adults was lower following vaccination with FluMist in comparison to the serum HAI antibody response following vaccination with TIV (Table 31). FluMist induced a serum antibody response rate of 29%, 39%, and 10% to the H1N1, H3N2, and B strains, respectively. TIV induced a serum antibody response rate of 96%, 94%, and 92% to the H1N1, H3N2, and B strains, respectively. These immunogenicity results were within the range of those previously reported in published studies.

The relatively low serum response rate induced by FluMist was accompanied by a high level of protection (Table 31). For example, the 10% serum HAI antibody rate to the B strain following vaccination with FluMist was accompanied by 100% protection against challenge with wild-type influenza B.

Vaccination with FluMist or TIV compared to placebo resulted in a high level of protection against laboratory-documented influenza illness following challenge with wild-type influenza virus. The efficacy of FluMist against all three strains of influenza combined was 85% (95% CI: 28,100), which was similar to the 71% (95% CI: 2, 97) efficacy of TIV. A low rate of serum HAI responses did not predict low efficacy with FluMist.

Table 31
Immunogenicity and Efficacy Following a Single Dose of FluMist in Serosusceptible Adults (Study AV003)

Vaccine Strain	Serum HAI Response to Immunization						Response to Challenge		
	HAI 4-Fold Rise n/N (%)	95% CI[a]	Post-Vaccination HAI GMFR[b]	95% CI[a]	Post-Vaccination HAI Titer ≥1:32 n/N (%)	95% CI[a]	Laboratory-Documented Illness[c] n/N (%)	Percent Efficacy[d]	95% CI[a]
	FluMist Treatment Group								
H1N1	7/24 (29)	(13, 51)	2.4	(1.4, 4.0)	3/24 (13)	(3,32)	1/10 (10)	80	
H3N2	9/23 (39)	(20, 61)	2.9	(1.6, 5.1)	5/23 (22)	(7,44)	1/9 (11)	78	
B	1/10 (10)	(0, 45)	1.3	(0.9, 1.9)	1/10 (10)	(0,45)	0/10 (0)	100	
							Combined	85	(28,100)
	TIV Treatment Group								
H1N1	22/23 (96)	(78, 100)	66.0	(34.3, 126.9)	22/23 (96)	(78,100)	2/10 (20)	60	
H3N2	16/17 (94)	(71, 100)	14.2	(7.6, 26.3)	12/17 (71)	(44,90)	2/12 (18)	64	
B	11/12 (92)	(62, 100)	20.2	(7.7, 52.8)	11/12 (92)	(62,100)	0/10 (0)	100	
							Combined	71	(2, 97)
	Placebo Treatment Group								
H1N1	5/24 (21)	(7, 42)	2.4	(1.2, 4.9)	5/24 (21)	(7,42)	6/12 (50)		
H3N2	2/18 (11)	(1, 35)	1.6	(0.8, 3.4)	2/18 (11)	(1,35)	4/8 (50)		
B	0/11 (0)	(0, 28)	1.1	(0.9, 1.4)	0/11 (0)	(0,28)	4/11 (36)		

Note: Serosusceptible adults defined as participants with pre-vaccination serum HAI titers of ≤1:8.

[a] Ninety-five percent confidence intervals refer to the rate difference of FluMist to Placebo, or TIV to Placebo.

[b] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[c] Laboratory documented illness indicates illness and viral shedding, or illness and ≥ four-fold rise in HAI titer following challenge.

[d] Efficacy was defined as protection against laboratory documented illness.

6.2.4. Conclusions from Immunogenicity Studies with FluMist in Adults

In the adult challenge trial (Study AV003), fewer than 40% of the serosusceptible adult participants developed a serum HAI antibody response following vaccination with FluMist. In spite of these low immune responses, vaccination with FluMist conferred 85% protection against laboratory-documented influenza illness following challenge with wild-type H1N1, H3N2, and B strains. These results support the following conclusions for adults:

- FluMist is efficacious against the three wild-type strains represented in the vaccine despite a low rate of serum antibody response.
- Immunological mechanisms, in addition to serum HAI antibody, contribute to protection following vaccination with FluMist.
- Immunity induced by FluMist appears to differ from that of TIV, for which HAI titers are considered to provide a useful correlate of protection.

6.3. Immunogenicity of FluMist in Children

6.3.1. Study Designs and Demographics

Aviron has completed six studies with immunogenicity endpoints in children. These studies have enrolled 1295 children for immunogenicity analyses. Approximately half of all children enrolled were White/Caucasian, approximately one-third were Hispanic and approximately 10% were Black. There was balance in the study population with respect to gender (53% male). The mean age of participants was 32 months and 36 months of age, FluMist versus placebo recipients, respectively. Three unique trivalent strain formulations of FluMist were administered in these studies (Table 32).

Table 32
Studies in Children with Immunogenicity Endpoints

Study	Number Enrolled	Vaccine Strain Formulation	Dose Regimen
AV002[a], AV002-2[a]	356	A/Texas/36/91 (H1N1) A/Johannesburg/33/94-like (H3N2) B/Panama/45/90	One
AV006 Year One	203[b]	A/Texas/36/91 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	One or Two
Year Two	160[b]	A/Shenzhen/227/95 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	One
AV011	222[c]	Challenge: A/Shenzhen/227/95 (H1N1) monovalent vaccine	One
AV007	500[b]	A/Texas/36/91 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	Two
		A/Shenzhen/227/95 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	Two
AV014	135	A/Shenzhen/227/95 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	Two
	90	A/Shenzhen/227/95 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like	Two

[a] Studies AV002 and AV002-2 were conducted under separate protocols in the United States and Chile, respectively.

[b] Number with data in the immunogenicity substudy.

[c] Immunogenicity/challenge substudy. Five to eight months following vaccination with vaccine containing:A/Shenzhen/227/95 (H1N1) A/Wuhan/359/95 (H3N2) B/Harbin/7/94-like, a subset of children enrolled in Study AV006 Year Two were challenged with monovalent H1N1.

6.3.2. Dose Escalation Studies

Analysis of serum HAI data indicated that there was a dosage-dependent serum HAI antibody response to each of the three vaccine strains following a single vaccination with FluMist. Increasing dosages of FluMist resulted in a higher percentage of participants who developed a ≥4-fold rise in HAI antibody titer following vaccination (Table 33, Table 34, and Table 35). This pattern was also evident when the post-vaccination GMFR in antibody titer and the proportion of participants who developed serum HAI titers ≥ 1:32 were analyzed (Table 33, Table 34, and Table 35). The dose-dependent effect to a single dose was most apparent in seronegative

participants. Among seronegative vaccinees who received a 10^6 or 10^7 $TCID_{50}$ dosage, 91% (40/44) seroconverted to the H3N2 strain, and 86% (38/44) developed a serum HAI titer ≥1:32 (Table 34). Forty-eight percent (26/54) of seronegative vaccinees seroconverted to the B strain, and 19% (10/54) developed a serum HAI titer ≥1:32 (Table 35).

Among seronegative children, only the 10^7 $TCID_{50}$ dosage of the H1N1 strain was immunogenic compared to lower dosages [16% of seronegative children (6/37) seroconverted following a single dose of FluMist, and 5% (2/37) had post-vaccination titers ≥1:32 to the H1N1 strain]. These results influenced the subsequent decision to administer two doses of FluMist in the Pediatric Protective Efficacy Trial, Study AV006, in order to induce an immune response to the H1N1 vaccine strain in a higher proportion of children.

In conclusion, results of these studies indicated that there was a dose-dependent response to a single dose of FluMist in children, with the highest response generally occurring at the highest dosage.

Table 33
Immunogenicity of the H1N1 Strain for Children Following a Single Dose of FluMist by Vaccine Dosage (Study AV002 and AV002-2)

Vaccine Dosage ($TCID_{50}$)	Pre-Vaccination Serostatus[a] to the H1N1 Strain	Evaluation of Immune Response Following Vaccination		
		Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	Post-Vaccination GMFR[b] (95% CI)[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)
10^4	Seronegative	0/18 (0)	1.0 (1.0, 1.0)	0/18 (0)
	All	0/18 (0)	1.0 (1.0, 1.0)	0/18 (0)
10^5	Seronegative	0/17 (0)	1.0 (1.0, 1.0)	0/17 (0)
	All	0/19 (0)	1.0 (1.0, 1.0)	2/19 (11)
10^6	Seronegative	0/38 (0)	1.0 (1.0, 1.0)	0/38 (0)
	All	0/54 (0)	1.0 (1.0, 1.1)	15/54 (28)
10^7	Seronegative	6/37 (16)	1.4 (1.1, 1.8)	2/37 (5)
	All	8/60 (13)	1.4 (1.2, 1.7)	25/60 (42)
Placebo	Seronegative	0/52 (0)	1.0 (1.0, 1.0)	0/52 (0)
	All	0/79 (0)	1.0 (1.0, 1.1)	25/80 (31)

Note: Data from Studies AV002 and AV002-2 for all children who received 10^6 or 10^7 $TCID_{50}$ dosages of spray or nasal drops in US and nasal spray in Chile were combined. Participants who received 10^4 and 10^5 doses and corresponding placebo in US sites were excluded from the H1N1 analysis because wild-type H1N1 circulated at the time of enrollment.

[a] Serostatus to the H1N1 strain was defined by pre-vaccination serum HAI titer. Children with an HAI titer ≤1:4 were classified as seronegative, and those with a titer ≥1:8 were classified as seropositive. "All" indicates all children, regardless of pre-vaccination serostatus.

[b] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[c] 95% CI indicates 95% confidence interval.

Table 34
Immunogenicity of the H3N2 Strain for Children Following a Single Dose of FluMist by Vaccine Dosage (Study AV002 and AV002-2)

Vaccine Dosage ($TCID_{50}$)	Pre-Vaccination Serostatus[a] to the H3N2 Strain	Evaluation of Immune Response Following Vaccination		
		Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	Post-Vaccination GMFR[b] (95% CI)[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)
10^4	Seronegative	5/16 (31)	3.0 (1.2, 7.5)	4/16 (25)
	All	6/57 (11)	1.3 (1.0, 1.8)	43/57 (75)
10^5	Seronegative	12/17 (71)	14.7 (5.5, 39.7)	11/17 (65)
	All	13/53 (25)	2.7 (1.7, 4.3)	46/53 (87)
10^6	Seronegative	21/23 (91)	48.8 (26.5, 89.9)	21/23 (91)
	All	23/54 (43)	6.2 (3.5, 10.9)	52/54 (96)
10^7	Seronegative	19/21 (90)	35.3 (18.8, 66.6)	17/21 (81)
	All	24/60 (40)	4.3 (2.6, 6.9)	55/60 (92)
Placebo	Seronegative	2/35 (6)	1.1 (0.9, 1.5)	1/35 (3)
	All	4/118 (3)	1.1 (1.0, 1.2)	81/119 (68)

Note: Data from Studies AV002 and AV002-2 for all children who received vaccine by nasal drops or spray in US and nasal spray in Chile were combined.

[a] Serostatus to the H3N2 strain was defined by pre-vaccination serum HAI titer. Children with an HAI titer ≤1:4 were classified as seronegative, and those with a titer ≥1:8 were classified as seropositive. "All" indicates all children, regardless of pre-vaccination serostatus.

[b] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[c] 95% CI indicates 95% confidence interval.

Table 35
Immunogenicity of B Strain for Children Following a Single Dose of FluMist by Vaccine Dosage (Study AV002 and AV002-2)

Vaccine Dosage ($TCID_{50}$)	Pre-Vaccination Serostatus[a] to the B Strain	Evaluation of Immune Response Following Vaccination		
		Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	Post-Vaccination GMFR[b] (95% CI)[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)
10^4	Seronegative	3/34 (9)	1.3 (0.9, 1.7)	1/34 (3)
	All	3/57 (5)	1.2 (1.0, 1.4)	22/57 (39)
10^5	Seronegative	14/32 (44)	3.2 (1.9, 5.3)	10/32 (31)
	All	16/53 (30)	2.4 (1.6, 3.4)	29/53 (55)
10^6	Seronegative	13/28 (46)	3.1 (1.8, 5.3)	5/28 (18)
	All	17/54 (31)	2.1 (1.5, 2.8)	29/54 (54)
10^7	Seronegative	13/26 (50)	3.0 (1.8, 4.9)	5/26 (19)
	All	15/60 (25)	1.9 (1.5, 2.4)	39/60 (65)
Placebo	Seronegative	0/74 (0)	1.0 (1.0, 1.0)	0/74 (0)
	All	0/118 (0)	0.9 (0.9, 1.0)	40/120 (33)

Note: Data from Studies AV002 and AV002-2 for all children who received vaccine by nasal drops or spray in US and nasal spray in Chile were combined.

[a] Serostatus to the B strain was defined by pre-vaccination serum HAI titer. Children with an HAI titer ≤1:4 were classified as seronegative, and children with an HAI titer ≥1:8 were classified as seropositive. "All" indicates all children, regardless of pre-vaccination serostatus.

[b] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[c] 95% CI indicates 95% confidence interval.

6.3.2.1. One-Dose Primary Regimen

A single dose of FluMist induced an immune response in 14% and 41% of children to the Texas and Shenzhen H1N1 strains, respectively, suggesting that immunogenicity could vary in a strain-dependent manner (Table 36). For each H1N1 strain tested, immunogenicity was the same for seronegative and all children.

A single dose of FluMist induced somewhat higher antibody response rates to the H3N2 and B strains (Table 37 and Table 38), and immunogenicity was higher in seronegative children, as had been observed previously in studies of CAIV. Among seronegative children there was a high HAI antibody response rate to each of the H3N2 strains (91% to the Johannesburg-like strain, and 92% to the Wuhan strain, Table 37). Analysis of two additional endpoints confirmed this result. First, among seronegative children the GMFR in antibody titer to the Johannesburg-

like and Wuhan H3N2 strains was 42-fold and 24-fold, respectively. Second, 85% or more of all children had HAI titers ≥ 1:32 to the H3N2 strains following vaccination (Table 37). Thus, analysis of the serum HAI data indicated that a single dose of FluMist was highly immunogenic in children with respect to H3N2 strains.

A single dose of FluMist induced serum antibody responses to the B/Panama and B/Harbin-like strains in 48% and 84% of seronegative children (Table 38), and immunogenicity of B strains was higher in seronegative children than in all children.

In summary, immunogenicity of a single primary dose of FluMist in seronegative children was higher for the H3N2 and B strains than for the H1N1 strains. For the H1N1 and B strains, immunogenicity appeared to depend on the vaccine strain. This was not entirely unanticipated, since strain-dependent immunogenicity of CAIV has been documented previously. Nevertheless, the results provided the impetus to determine if vaccination with two primary doses of FluMist in children could increase the response rate to the three constituent strains.

6.3.2.2. Two-Dose Primary Regimen

Vaccination of children with two doses of FluMist was tested to determine if two doses could produce a superior antibody response to the three constituent vaccine strains. Aggregate analysis of the results of two-dose regimens (Studies AV006 Year One, AV007, and AV014) is presented in Table 39, Table 40, and Table 41. For each of the three vaccine strains, the immune response following two doses was higher than following a single dose.

The advantage of two vaccine doses was most striking for the H1N1 strain among seronegative children. For example, the seroconversion rate among seronegative children to the Shenzhen H1N1 strain was 41% following a single dose of FluMist, and 82% following two doses (compare Table 36 with Table 39). Likewise, the response to the Texas H1N1 strain increased from 14% to 51%. The seroconversion rate increased from 92% to 99% for the Wuhan H3N2 strain and from 84% to 99% for the B/Harbin-like vaccine strain when two doses of FluMist were administered (compare Table 37 with Table 40, and Table 38 with Table 41). Furthermore, the serum HAI antibody GMFR and the proportion of vaccinees that developed titers ≥1:32 were higher when two doses of vaccine were administered.

The superior antibody response to two doses was also evident among all children regardless of serostatus. Specifically, the serum HAI antibody response rate to the Shenzhen H1N1 strain was 41% following a single dose, and 78% following two doses of FluMist (compare Table 36 with Table 39). Among all participants, the immune response rate increased from 52% to 62%

for the Wuhan H3N2 strain, and from 65% to 73% for the B/Harbin-like vaccine strain when two doses of FluMist were administered (compare Table 37 with Table 40, and Table 38 with Table 41). Serum HAI antibody GMFR and the proportion of vaccinees that developed titers ≥1:32 were also higher following two primary doses of vaccine.

In summary, comparison with a one-dose primary vaccination, two primary doses induced a higher serum antibody response rate in both seronegative and all children to all three constituent vaccine strains.

6.3.2.3. Conclusions from Immune Response to One- and Two-Dose Regimens

Results of one- and two-dose regimen studies with FluMist were consistent with those obtained in previous studies with CAIV in young seronegative children. This includes a study of monovalent CAIV which indicated that one dose stimulated a systemic antibody response in 90% of seronegative children whereas two doses stimulated a response in 100% of vaccinees, suggesting that improved immunogenicity in seronegative children might be achieved by a two-dose regimen of CAIV. In a study of trivalent CAIV in seronegative children, the serum HAI antibody response rate following a single dose was 12%, 88%, and 18% to the H1N1, H3N2, and B vaccine strains, respectively. Administration of a second dose of vaccine to these children resulted in an 80%, 93%, and 67% cumulative antibody response rate to the H1N1, H3N2, and B components, respectively. Administration of a third dose did not significantly increase the cumulative response rate to any of the three CAIV strains.

A two-dose primary vaccination regimen is also supported by experience with multivalent live vaccines and inactivated influenza vaccine, which are administered in multiple doses in order to stimulate an immune response to all the component strains. The requirement for multiple vaccine doses in young children is presumably due to a combination of host-related and vaccine strain-related factors. For example, young children may not have been exposed to some of the influenza subtypes contained in the vaccine and, consequently, may be seronegative or immunologically unprimed to certain subtypes. In addition, it is possible that certain vaccine strains are favored in that they replicate better in the host and thereby induce an immune response following the first vaccine dose. Immunity that exists at the time the second dose of vaccine is administered may suppress the replication of the previously favored strains, allowing less efficiently replicating strains to stimulate an immune response following the second dose.

In summary, the rationale for a two-dose primary regimen of FluMist in children is supported by:

- immunogenicity data for one- and two-dose FluMist regimens.

- previous multidose studies with CAIV, other live vaccines, and inactivated influenza vaccine.
- epidemiological data showing the age-related acquisition of immunity to influenza strains.

Table 36
Immune Response of Children to H1N1 Following a Single 10^6 or 10^7 $TCID_{50}$ Dose of FluMist

Treatment Group	H1N1 FluMist Strain[a]	Pre-Vaccination Serostatus[b]	Evaluation of Immune Response Following Vaccination					
			Serum HAI Antibody Response: 4-Fold Rise n/N (%)	95% CI[c]	Post-Vaccination GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥ 1:32 n/N (%)	95% CI[c]
FluMist	A/Shenzhen	Seronegative	113/273 (41)	(35,47)	2.6	(2.3, 3.0)	35/273 (13)	(9,17)
	A/Texas		24/176 (14)	(9,20)	1.4	(1.2, 1.6)	10/176 (6)	(3, 10)
	A/Shenzhen	All	113/278 (41)	(35, 47)	2.6	(2.3, 3.0)	41/279 (15)	(11, 19)
	A/Texas		38/263 (14)	(10, 19)	1.5	(1.3, 1.6)	92/263 (35)	(29, 41)
Placebo		Seronegative	0/98 (0)	(0, 4)	1.0	(1.0, 1.0)	0/98 (0)	(0, 4)
		All	0/139 (0)	(0, 3)	1.0	(1.0, 1.0)	34/140 (24)	(17, 32)

Note: Data Integrated for serum HAI response to H1N1 strain following one 10^6 or 10^7 $TCID_{50}$ dose of FluMist in Studies AV002, AV002-2, AV006 Year One, AV007 and AV014.

[a] H1N1 vaccine strains used were A/Texas/36/91 and A/Shenzhen/227/95.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

Table 37
Immune Response of Children to H3N2 Following a Single 10^6 or 10^7 $TCID_{50}$ Dose of FluMist

Treatment Group	H3N2 FluMist Strain[a]	Pre-Vaccination Serostatus[b]	Evaluation of Immune Response Following Vaccination					
			Serum HAI Antibody Response: 4-Fold Rise n/N (%)	95% CI[c]	Post-Vaccination GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥ 1:32 n/N (%)	95% CI[c]
FluMist	A/Johannesburg-like	Seronegative	40/44 (91)	(78, 97)	41.8	(27.4, 63.9)	38/44 (86)	(73, 95)
	A/Wuhan		186/203 (92)	(87, 95)	23.9	(20.0, 28.4)	159/203 (78)	(72, 84)
	A/Johannesburg-like	All	47/114 (41)	(32, 51)	5.1	(3.5, 7.4)	107/114 (94)	(88, 97)
	A/Wuhan		224/428 (52)	(47, 57)	5.5	(4.7, 6.5)	365/429 (85)	(81, 88)
Placebo		Seronegative	3/53 (6)	(1, 16)	1.2	(1.0, 1.4)	2/53 (4)	(0, 13)
		All	4/139 (3)	(1, 7)	1.1	(1.0, 1.2)	75/140 (54)	(45, 62)

Note: Data Integrated for serum HAI response to H3N2 strain following one dose of 10^6 or 10^7 $TCID_{50}$ of FluMist in Studies AV002, AV002-2, AV006 Year One, AV007, and AV014.

[a] H3N2 vaccine strains used were A/Johannesburg/33/94-like and A/Wuhan/359/95.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

Table 38
Immune Response of Children to Influenza B Following a Single 10^6 or 10^7 $TCID_{50}$ Dose of FluMist

Treatment Group	B FluMist Strain[a]	Pre-Vaccination Serostatus[b]	Evaluation of Immune Response Following Vaccination					
			Serum HAI Antibody Response: 4-Fold Rise n/N (%)	95% CI[c]	Post-Vaccination GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥ 1:32 n/N (%)	95% CI[c]
FluMist	B/Panama	Seronegative	26/54 (48)	(34, 62)	3.1	(2.1, 4.3)	10/54 (19)	(9, 31)
	B/Harbin		264/315 (84)	(79, 88)	7.5	(6.7, 8.3)	107/315 (34)	(29, 39)
	B/Panama	All	32/114 (28)	(20, 37)	2.0	(1.6, 2.4)	68/114 (60)	(50, 69)
	B/Harbin		277/428 (65)	(60, 69)	4.9	(4.4, 5.4)	200/429 (47)	(42, 51)
Placebo	[redacted]	Seronegative	0/83 (0)	(0,4)	1.0	(1.0, 1.0)	0/83 (0)	(0, 4)
		All	0/139 (0)	(0,3)	0.9	(0.9, 1.0)	32/140 (23)	(16, 31)

Note: Data Integrated for serum HAI response to B strain following one 10^6 or 10^7 $TCID_{50}$ dose of FluMist in Studies AV002, AV002-2, AV006 Year One, AV007, and AV014.

[a] Type B vaccine strains used were B/Panama/45/90 and B/Harbin/7/94-like.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

Table 39
Immune Response of Children to Influenza H1N1 Following Two Primary 10^7 $TCID_{50}$ Doses of FluMist

Treatment Group	H1N1 FluMist Strain[a]	Pre-Vaccination Serostatus to H1N1 Strain[b]	Evaluation of Immune Response Following Vaccination					
			Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	95% CI[c]	Post-Vaccination GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)	95% CI[c]
FluMist	A/Shenzhen	Seronegative	389/476 (82)	(78, 85)	9.6	(8.5, 10.7)	231/476 (49)	(44, 53)
	A/Texas		78/153 (51)	(43, 59)	3.4	(2.8, 4.2)	34/153 (22)	(16, 30)
	A/Shenzhen	All	389/500 (78)	(74, 81)	8.6	(7.7, 9.7)	256/501 (51)	(47, 56)
	A/Texas		90/209 (43)	(36, 50)	3.0	(2.5, 3.5)	85/209 (41)	(34,48)
Placebo	[redacted]	Seronegative	3/125 (2)	(0, 7)	1.0	(1.0, 1.1)	0/125 (0)	(0, 3)
		All	4/149 (3)	(1,7)	1.0	(0.9, 1.1)	17/149 (11)	(7, 18)

Note: Data integrated for serum HAI response to H1N1 strain following two doses of FluMist formulated to contain 10^7 $TCID_{50}$ in Studies AV006 Year One, AV007, and AV014.

[a] The A/Texas/36/91 H1N1 strain was used in studies AV006 Year One and in AV007, whereas the A/Shenzhen/227/95 H1N1 was used in studies AV007 and AV014.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

Table 40
Immune Response of Children to Influenza H3N2 Following Two Primary 10^7 $TCID_{50}$ Doses of FluMist

Treatment Group	H3N2 FluMist Strain[a]	Pre-Vaccination Serostatus to H3N2 Strain[b]	Evaluation of Immune Response Following Two Vaccine Doses					
			Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	95% CI[c]	Post-Dose Two GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)	95% CI[c]
FluMist	A/Wuhan	Seronegative	388/391 (99)	(98, 100)	34.0	(31.4, 36.9)	361/391 (92)	(89, 95)
		All	438/709 (62)	(58, 65)	8.6	(7.6, 9.8)	668/710 (94)	(92, 96)
Placebo		Seronegative	6/83 (7)	(3, 15)	1.3	(1.1, 1.7)	6/83 (7)	(3, 15)
		All	9/147 (6)	(3, 11)	1.2	(1.1, 1.4)	60/147 (41)	(33, 49)

Note: Data integrated for serum HAI response to H3N2 strain following two doses of FluMist formulated to contain 10^7 $TCID_{50}$ in Studies AV006 Year One, AV007, and AV014.

[a] All studies used the A/Wuhan/359/95 H3N2 vaccine strain.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

Table 40
Immune Response of Children to Influenza H3N2 Following Two Primary 10^7 $TCID_{50}$ Doses of FluMist

Treatment Group	H3N2 FluMist Strain[a]	Pre-Vaccination Serostatus to H3N2 Strain[b]	Evaluation of Immune Response Following Two Vaccine Doses					
			Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	95% CI[c]	Post-Dose Two GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)	95% CI[c]
FluMist	A/Wuhan	Seronegative	388/391 (99)	(98, 100)	34.0	(31.4, 36.9)	361/391 (92)	(89, 95)
		All	438/709 (62)	(58, 65)	8.6	(7.6, 9.8)	668/710 (94)	(92, 96)
Placebo		Seronegative	6/83 (7)	(3, 15)	1.3	(1.1, 1.7)	6/83 (7)	(3, 15)
		All	9/147 (6)	(3, 11)	1.2	(1.1, 1.4)	60/147 (41)	(33, 49)

Note: Data Integrated for serum HAI response to H3N2 strain following two doses of FluMist formulated to contain 10^7 $TCID_{50}$ in Studies AV006 Year One, AV007, and AV014.

[a] All studies used the A/Wuhan/359/95 H3N2 vaccine strain.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

Table 41
Immune Response of Children to Influenza B Following Two Primary 10^7 $TCID_{50}$ Doses of FluMist

Treatment Group	B FluMist Strain[a]	Pre-Vaccination Serostatus to B Strain[b]	Evaluation of Immune Response Following Two Vaccine Doses					
			Serum HAI Antibody Response: ≥4-Fold Rise n/N (%)	95% CI[c]	Post-Dose Two GMFR[d]	95% CI[c]	Post-Vaccination HAI Titer ≥1:32 n/N (%)	95% CI[c]
FluMist	B/Harbin	Seronegative	464/469 (99)	(98, 100)	16.8	(15.7, 17.9)	349/469 (74)	(70, 78)
		All	520/709 (73)	(70, 77)	8.0	(7.3, 8.8)	555/710 (78)	(75, 81)
Placebo		Seronegative	2/88 (2)	(0, 8)	1.1	(1.0, 1.1)	0/88 (0)	(0, 4)
		All	3/149 (2)	(0, 6)	1.1	(1.0, 1.1)	33/149 (22)	(16, 30)

Note: Data Integrated for serum HAI response to B strain following two doses of FluMist formulated to contain 10^7 $TCID_{50}$ in Studies AV006 Year One, AV007, and AV014.

[a] All studies used the B/Harbin/7/94-like vaccine strain.

[b] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[c] 95% CI indicates 95% confidence interval.

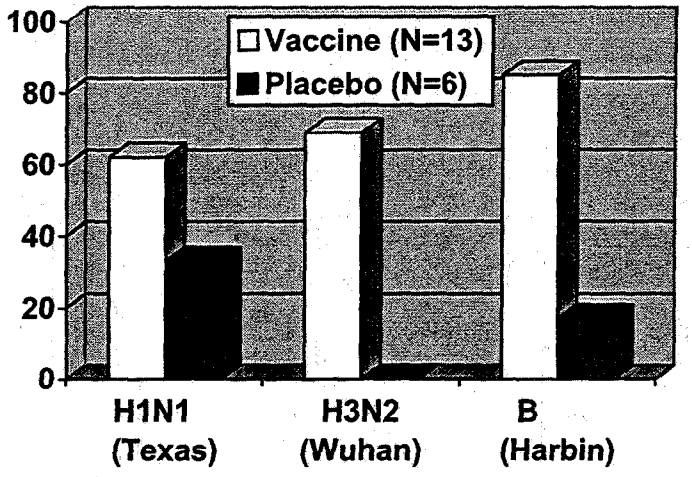
[d] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

6.3.2.4. Nasal IgA Antibody Data

Nasal wash specimens were obtained during Year One and Year Two of Study AV006 from a subset of children enrolled at a single clinical site (Vanderbilt University, Nashville). In Year One, specimens were obtained from 19 children (13 vaccinees and six placebo recipients) who were vaccinated with two doses of FluMist or placebo, and in Year Two, specimens were obtained from 13 participants who had been re-vaccinated with a single dose. Specimens were analyzed at Vanderbilt University for influenza-specific nasal IgA antibody responses. An immune response was defined as either conversion in the ELISA assay from negative to positive, or a ≥4-fold rise in post-vaccination nasal IgA level compared to pre-vaccination nasal IgA level.

For these 13 participants, 62%, 69%, and 85% had a nasal IgA antibody response to the H1N1, H3N2, and B strains, respectively, following two doses of FluMist (Figure 1). The response rates to the H3N2 and B strains were clearly higher among vaccine recipients compared to placebo recipients. In contrast, 33% of placebo recipients developed a nasal IgA response to the H1N1 strain, in comparison to 61% of vaccine recipients. The results indicate that all three strains in the FluMist formulation induced nasal IgA antibodies following two doses of vaccine.

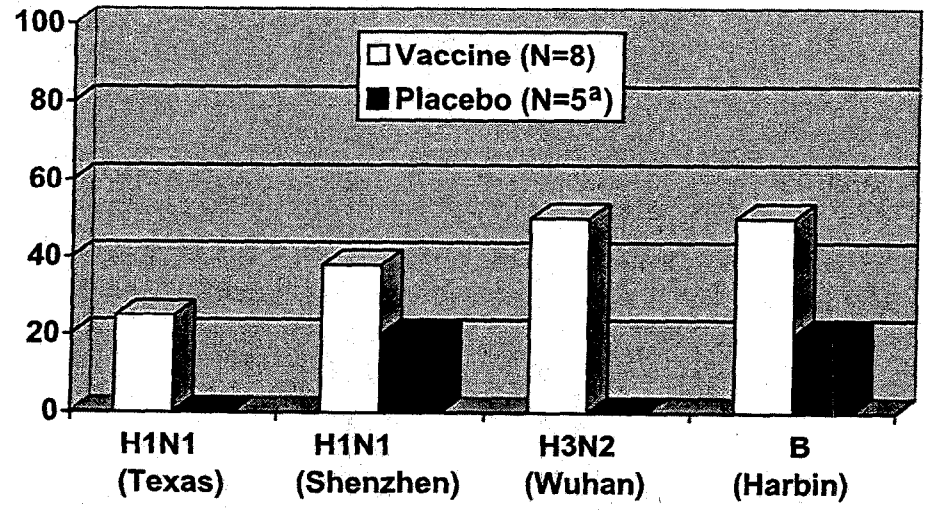
Figure 1
Proportion of Children with Strain-Specific Anti-HA Nasal IgA Antibody Response Following Two Doses of FluMist in Study AV006 Year One (Vanderbilt Site Only)



Note: A nasal IgA response was defined as either conversion from negative to positive, or as a ≥4-fold rise post-Dose Two among children who were positive pre-vaccination.

Nasal antibody to the three strains remained detectable prior to re-vaccination in Year Two. Because the A/Shenzhen/227/95 (H1N1) vaccine component was substituted in Year Two for the A/Texas/36/91 (H1N1) strain, IgA responses were measured to both strains. The results indicated that 25% and 38% of children developed a nasal IgA antibody response to the Texas and Shenzhen H1N1 strains, respectively, and 50% responded to the H3N2 and B vaccine strains (Figure 2).

Figure 2
Proportion of Children with Strain-Specific Anti-HA Nasal IgA Antibody Response Following Re-Vaccination with FluMist in Year Two (Vanderbilt Site Only)



Note: A nasal IgA response was defined as either conversion from negative to positive, or as a ≥4-fold rise after re-vaccination among children who were positive before re-vaccination.

[a] Excludes one placebo recipient who had influenza in Year One.

In summary, primary vaccination with FluMist stimulated a nasal IgA antibody response to the three vaccine strains in a small subset of children tested. Prior to re-vaccination during the second year of the study, most vaccinees had detectable nasal IgA antibody. Re-vaccination with FluMist induced a rise in nasal IgA antibody in 25% to 50% of these children. Although efficacy could not be demonstrated against community-acquired H1N1 virus since H1N1 strains did not circulate, the comparable nasal IgA responses to the three FluMist strains predict that the children may have been protected against H1N1 strains. This prediction was confirmed in Study AV011, in which prior vaccination of children with FluMist provided 83% efficacy against viral shedding following challenge with monovalent H1N1 vaccine virus.

6.3.2.5. Heterotypic Antibody Data

One of the potential advantages of a live influenza vaccine is that it might be expected to stimulate broad immunity against antigenically drifted strains. The basis for this expectation is that a live replicating vaccine virus would present to the immune system a complete complement of influenza virus antigens in their native conformation. As a result, neutralizing antibodies could be produced to both protective antigens, the hemagglutinin and neuraminidase.

On occasion, it has been difficult to predict precisely which antigens should be included in the influenza vaccine. For example, the H3N2 strain recommended for the 1997–1998 influenza vaccine (A/Wuhan/359/95) was not a good antigenic match with the predominant H3N2 wild-type strain circulating during the 1997–1998 influenza season (A/Sydney/05/97).

To address the issue of whether FluMist stimulated immunity that was cross-reactive with antigenically drifted strains, serum specimens obtained during Year One of the Pediatric Protective Efficacy Trial (Study AV006) were tested in HAI assays against several H3N2 strains isolated during influenza seasons immediately preceding or following Year One of Study AV006. Antigenic characterization performed by the CDC using strain-specific ferret antisera indicated that the strains included in the analysis represented a spectrum of H3N2 antigenic variants. The A/Sydney/05/97 (H3N2) strain was of particular interest because it was the predominant H3N2 strain circulating during the 1997–1998 influenza season and had undergone significant antigenic drift from the H3N2 vaccine strain A/Wuhan/359/95.

Children vaccinated with FluMist developed serum HAI antibodies that cross-reacted with H3N2 strains that circulated either before or after the Wuhan strain (Table 42). Specifically, following a single dose of FluMist, at least 84% of seronegative children seroconverted to the Thessalonika, Nanchang, Wuhan, and Sydney strains, and 36% seroconverted to the Russia and Johannesburg strains. Following a second dose of FluMist, most children seroconverted to all six of the H3N2 strains tested. In spite of the antigenic difference between the Sydney and Wuhan strains, the data indicated that 96% of seronegative children immunized with FluMist containing the Wuhan strain developed a serum antibody response that cross-reacted with the Sydney strain.

The serological results of Study AV006 Year One were confirmed by the efficacy results obtained during Year Two, in which FluMist containing the A/Wuhan/359/95 strain was 85.9% efficacious against culture-confirmed influenza A/Sydney/05/97, an H3N2 drifted strain. Previous studies conducted prior to Aviron sponsorship, have also documented heterotypic efficacy of CAIV against antigenically drifted strains.

Table 42
Development of Cross-Reactive H3N2 Antibodies for Seronegative Children
(Study AV006 Year One)

Seroconversion Among FluMist Recipients Seronegative[a] To:						
	Wuhan/359/95[b]	**Sydney/05/97**	**Nanchang/933/95**	**Thessalonika/1/95**	**Russia/269/95**	**Johannesburg/33/94**
	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]
Post-Dose One	23/25 (92) (74, 99)	23/24 (96) (79, >99)	23/26 (88) (70, 98)	21/25 (84) (64, 95)	9/25 (36) (18, 57)	9/25 (36) (18, 57)
Post-Dose Two	24/25 (96) (80, >99)	23/24 (96) (79, >99)	25/26 (96) (80, >99)	24/25 (96) (80, >99)	18/23 (78) (56, 93)	13/25 (52) (31, 72)
Seroconversion Among Placebo Recipients Seronegative[a] To:						
	Wuhan/359/95[b]	**Sydney/05/97**	**Nanchang/933/95**	**Thessalonika/1/95**	**Russia/269/95**	**Johannesburg/33/94**
	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]	n/N (%) 95% CI[c]
Post-Dose One	0/15 (0) (0, 18)	1/14 (7) (0, 34)	1/16 (6) (0, 30)	0/14 (0) (0, 19)	0/14 (0) (0, 19)	0/15 (0) (0, 18)
Post-Dose Two	1/15 (7) (0, 32)	2/14 (14) (2, 43)	2/16 (13) (2, 38)	1/14 (7) (0, 34)	1/14 (7) (0, 34)	1/15 (7) (0, 32)

Note: These serum HAI assays were conducted at Vanderbilt University.

[a] Children who were seronegative to each individual H3N2 strain prior to vaccination are included.

[b] The H3N2 vaccine strain contained in FluMist vaccine used in Study AV006 Year One was A/Wuhan/359/95 (H3N2).

[c] 95% CI indicates 95% confidence interval.

6.3.2.6. Correlates of Protection

Specific immune responses to CAIV have been linked with protection of children or adults against influenza. Briefly, these studies concluded that antibodies specific for the viral HA and NA proteins were associated with protection, and that protective antibodies were present in either serum or nasal secretions. None of the previous studies of CAIV in children or adults have identified a single type of immune response that has both high *positive* and high *negative* predictive value for efficacy.

In Study AV011, protection against shedding of challenge monovalent H1N1 vaccine was used as a surrogate of FluMist efficacy in children. With the goal of identifying immune correlates of this protection, four measures of the immune response were evaluated in Study AV011. Serum HAI, serum IgG, serum neutralizing, and nasal IgA antibody responses were measured. Because results obtained in serum HAI and serum IgG ELISA assays were similar, only serum HAI, serum neutralization, and nasal IgA results are discussed herein.

Table 43 presents pre-challenge antibody status measured by serum HAI assay and nasal IgA assay prior to challenge with H1N1 monovalent vaccine and protection against shedding. The results indicated that among prior placebo participants, the presence of serum HAI antibody was significantly associated with protection from shedding virus (p=0.0001). Among prior FluMist recipients, the presence of nasal antibody was significantly associated with the protection from shedding challenge virus (p=0.0124).

Table 43
Pre-Challenge Antibody Status and Protection Against Challenge with H1N1 Vaccine Strain in Study AV011

Protection	Treatment Group in Study AV006			
	FluMist		Placebo	
	Serum HAI Negative (HAI Titer ≤1:4)	Serum HAI Positive (HAI Titer ≥1:8)	Serum HAI Negative (HAI Titer ≤1:4)	Serum HAI Positive (HAI Titer ≥1:8)
Shedding Post-Challenge	4/45 (9)	2/96 (2)	19/50 (38)	0/25 (0)
p-value[a]	0.0821		0.0001	
	Nasal IgA Negative n/N (%)	Nasal IgA Positive n/N (%)	Nasal IgA Negative n/N (%)	Nasal IgA Positive n/N (%)
Shedding Post-Challenge	5/41 (12)	1/88 (1)	16/44 (36)	3/23 (13)
p-value[a]	0.0124		0.0512	

Note: Children were vaccinated with one or two doses of FluMist or placebo prior to the 1996–1997 influenza season (Study AV006, Year One), and with one dose of FluMist or placebo prior to the 1997–1998 season (Study AV006, Year Two). Following vaccination in the 1997–1998 season, a subset of children (144 prior FluMist recipients and 78 prior placebo recipients) were challenged with monovalent CAIV, A/Shenzhen/227/95 (H1N1) (Study AV011).

[a] Fisher's Exact test.

In prior FluMist recipients, sixteen of 144 (11%) had neither serum HAI antibody nor nasal IgA at the time of challenge. Nevertheless, among the 16 prior vaccinated children with no detectable serum HAI or nasal IgA antibodies efficacy was 45% (95% CI: -22, 79). Twelve of these 16 children had detectable neutralizing antibody, which may have contributed to the observed efficacy in the absence of detectable serum HAI or nasal IgA antibody. These data suggest that vaccination with FluMist induces immune factors such as neutralizing antibody in addition to serum HAI antibody or nasal IgA antibody.

In summary, the data support the conclusion that there was no single correlate of protection for FluMist, and that serum HAI, serum neutralizing, and nasal IgA antibodies played a role in protection. Multiple mechanisms of protection induced in children by FluMist was not surprising in light of previous results with CAIV, in which both serum and nasal antibodies correlated with protection. The complex nature of the protective immune response to immunization with FluMist resembles that induced by natural infection with influenza.

6.3.3. Consistency of the Immune Response to FluMist

6.3.3.1. Clinical Consistency Lot Study

The clinical consistency lot trial (Study AV007) measured the serum HAI antibody response of children following immunization with two doses of FluMist. Children were immunized with one of three consistency lots, or with the FluMist vaccine used in the first year of the Pediatric Protective Efficacy Trial (referred to hereafter as the "Efficacy Vaccine"), or placebo. The strain formulation of the consistency lots differed from that of the Efficacy Vaccine with respect to the H1N1 component: consistency lots contained the A/Shenzhen/227/95 (H1N1) strain whereas the Efficacy Vaccine contained the A/Texas/36/91 (H1N1) strain. Tests performed by the Influenza Branch at the CDC indicated that the A/Shenzhen/227/95 (H1N1) vaccine strain was antigenically well matched both to the A/Texas/36/91 (H1N1) strain and to other currently circulating H1N1 strains.

Serum specimens from children vaccinated with any of the three consistency lots or Efficacy Vaccine in Study AV007 were tested in HAI assays using homologous virus strains. Thus, serum specimens from children vaccinated with consistency lots were tested in HAI assays using the A/Shenzhen/227/95 (H1N1) strain, whereas those obtained from children who received the Efficacy Vaccine were tested against the A/Texas/36/91 (H1N1) strain. In addition, in order to study serum HAI cross-reactivity responses, a subset of participants were tested against both H1N1 strains.

The primary immunogenicity objective of this study was to show that two doses of any of the three consistency lots produced an equivalent fold-rise in serum HAI titer. As pre-specified in the protocol, the lots were to be declared equivalent if the 95% CI for the strain-specific ratios of serum HAI antibody GMFR between the three lots fell within the interval bounded by 1/4 and 4.

Each of the three consistency lots was highly immunogenic and produced comparable HAI immune responses after two doses in vaccinated children regardless of pre-vaccination serostatus (Table 44, Table 45, and Table 46). Two doses of each of the consistency lots stimulated moderate to high levels of antibodies among seronegative children, resulting in a GMFR in serum HAI antibody ranging from 8.6 to 18.4 for the H1N1 strain, 38.7 to 43.0 for the H3N2 strain, and 15.2 to 20.2 for the B strain. Among all participants, the GMFR in serum HAI antibody ranged between 7.2 to 15.3 for the H1N1 strain, 10.7 to 16.7 for the H3N2 strain, and 6.5 and 7.4 for the B strain, relative to baseline GMT. Furthermore, regardless of which consistency lot they received, H1N1 seroconversion rates among seronegative children ranged

from 79% to 94%, H3N2 seroconversion rates ranged from 99% to 100%, and influenza B seroconversion rates ranged from 96% to 100%. The majority of seronegative children vaccinated with the consistency lots developed HAI titers ≥1:32 in response to H1N1, H3N2, and B strains. These results indicated that immunization of children with different vaccine lots containing the same H1N1, H3N2, and B strains produced a consistent immune response.

The Efficacy Vaccine was also compared in the consistency trial. The immune response rates and GMFR in antibodies to the H3N2 and B strains induced by the field trial vaccine were similar to those induced by the consistency lots (Table 44, Table 45, and Table 46). This result was expected, because the H3N2 and B strains were identical in the consistency lots and the field trial vaccine. In contrast, children vaccinated with the Efficacy Vaccine, which contained the A/Texas/36/91 (H1N1) strain, had lower serum HAI immune responses to the H1N1 component. It is important to note, however, that inclusion of a different H1N1 strain in the vaccine did not affect the immune response to the H3N2 and B strains.

Additional subgroup analyses of immunogenicity were performed. These analyses compared immunogenicity by gender, race, and time between vaccinations. The study was not powered to detect significant differences among subgroups. However, a trend toward development of a slightly higher GMT of serum HAI antibodies among female children and among children of Hispanic descent was noted. Neither age nor time between vaccinations appeared to affect the immune response.

In summary, each of the three consistency lots was highly immunogenic in vaccinated children, regardless of pre-vaccination serostatus. In accordance with the primary endpoint of this study, the strain-specific GMFR in antibody titer induced by the three consistency lots had a 95% confidence interval that fell within the interval bounded by 1/4 and 4. These results indicated that vaccination with any of the consistency lots resulted in a robust and consistent immune response to each of the three constituent vaccine strains.

Table 44
Immune Response of Children to H1N1 Following Two Doses of FluMist in Consistency Lot Study (Study AV007)

Evaluation of Immune Response Following Two Vaccine Doses								
Vaccine Lot	Strain	Pre-Vaccination Serostatus[a] to H1N1 Strain	Serum HAI Antibody Response: 4-Fold Rise n/N (%)	95% CI[b]	HAI GMFR[c]	95% CI[b]	Post-Vaccination HAI Titer ≥ 1:32 n/N (%)	95% CI[b]
Lot 1	A/Shenzhen	Seronegative	72/83 (87)	(78,93)	13.7	(10.5, 17.8)	50/83 (60)	(49, 71)
		All	72/92 (78)	(68, 86)	10.7	(8.1, 14.3)	60/93 (65)	(54, 74)
Lot 2	A/Shenzhen	Seronegative	83/88 (94)	(87,98)	18.4	(14.5, 23.4)	62/88 (70)	(60, 80)
		All	83/94 (88)	(80, 94)	15.3	(11.7, 20.0)	68/94 (72)	(62, 81)
Lot 3	A/Shenzhen	Seronegative	69/87 (79)	(69, 87)	8.6	(6.5, 11.3)	40/87 (46)	(35, 57)
		All	69/95 (73)	(63, 81)	7.2	(5.5, 9.5)	48/95 (51)	(40, 61)
Efficacy Vaccine[d]	A/Texas	Seronegative	33/79 (42)	(31, 53)	2.7	(2.1, 3.5)	12/79 (15)	(8, 25)
		All	35/94 (37)	(27, 48)	2.5	(2.0, 3.2)	25/94 (27)	(18, 37)
Placebo		Seronegative	2/85 (2)	(0, 8)	1.0	(1.0, 1.1)	0/85 (0)	(0, 4)
		All	2/91 (2)	(0, 8)	1.0	(0.9, 1.1)	5/91 (5)	(2, 12)

Note: HAI assays were performed using H1N1 antigens homologous to the vaccine strain administered. Thus, serum specimens obtained from children vaccinated with consistency lots or Efficacy Vaccine were tested against A/Shenzhen/227/95 or A/Texas/36/91, respectively.

[a] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[b] 95% CI indicates 95% confidence interval.

[c] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[d] FluMist used in Year One of Study AV006.

Table 45
Immune Response of Children to H3N2 Following Two Doses of FluMist in Consistency Lot Study (Study AV007)

Evaluation of Immune Response Following Two Vaccine Doses								
Vaccine Lot	Strain	Pre-Vaccination Serostatus[a] to H3N2 Strain	Serum HAI Antibody Response: 4-Fold Rise n/N (%)	95% CI[b]	HAI GMFR[c]	95% CI[b]	Post-Vaccination HAI Titer ≥ 1:32 n/N (%)	95% CI[b]
Lot 1	A/Wuhan	Seronegative	59/59 (100)	(94, 100)	40.5	(33.3, 49.2)	56/59 (95)	(86, 99)
		All	63/92 (68)	(58, 78)	13.3	(9.4, 18.7)	90/93 (97)	(91, 99)
Lot 2	A/Wuhan	Seronegative	67/68 (99)	(92, 100)	43.0	(35.9, 51.5)	67/68 (99)	(92, 100)
		All	68/94 (72)	(62, 81)	16.7	(11.9, 23.6)	91/94 (97)	(91, 99)
Lot 3	A/Wuhan	Seronegative	58/58 (100)	(94, 100)	38.7	(32.9, 45.6)	56/58 (97)	(88, 100)
		All	63/95 (66)	(56, 76)	10.7	(7.5, 15.2)	93/95 (98)	(93, 100)
Efficacy Vaccine[d]	A/Wuhan	Seronegative	59/59 (100)	(94, 100)	47.7	(40.5, 56.3)	59/59 (100)	(94, 100)
		All	61/94 (65)	(54, 74)	13.2	(9.2, 19.0)	94/94 (100)	(96, 100)
Placebo		Seronegative	4/57 (7)	(2, 17)	1.3	(1.0, 1.7)	4/57 (7)	(2, 17)
		All	6/91 (7)	(2, 14)	1.2	(1.0, 1.4)	37/91 (41)	(30, 51)

[a] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[b] 95% CI indicates 95% confidence interval.

[c] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[d] FluMist used in Year One of Study AV006.

Table 46
Immune Response of Children to Influenza B Following Two Doses of FluMist in Consistency Lot Study (Study AV007)

Evaluation of Immune Response Following Two Vaccine Doses								
Vaccine Lot	Strain	Pre-Vaccination Serostatus[a] to B Strain	Serum HAI Antibody Response: 4-Fold Rise n/N (%)	95% CI[b]	HAI GMFR[c]	95% CI[b]	Post-Vaccination HAI Titer ≥ 1:32 n/N (%)	95% CI[b]
Lot 1	B/Harbin	Seronegative	53/55 (96)	(87, 100)	15.2	(12.5, 18.5)	41/55 (75)	(61, 85)
		All	63/92 (68)	(58, 78)	6.7	(5.2, 8.7)	76/93 (82)	(72, 89)
Lot 2	B/Harbin	Seronegative	48/48 (100)	(93, 100)	20.2	(15.9, 25.6)	39/48 (81)	(67, 91)
		All	60/94 (64)	(53, 73)	6.5	(4.9, 8.6)	77/94 (82)	(73, 89)
Lot 3	B/Harbin	Seronegative	51/51 (100)	(93, 100)	18.6	(15.6, 22.1)	42/51 (82)	(69, 92)
		All	68/95 (72)	(61, 80)	7.4	(5.8, 9.5)	84/95 (88)	(80, 94)
Efficacy Vaccine[d]	B/Harbin	Seronegative	62/62 (100)	(94, 100)	14.3	(12.5, 16.9)	40/62 (65)	(51, 76)
		All	66/94 (70)	(60, 79)	6.5	(5.0, 8.4)	68/94 (72)	(62, 81)
Placebo		Seronegative	1/50 (2)	(0, 11)	1.0	(1.0, 1.1)	0/50 (0)	(0, 7)
		All	1/91 (1)	(0, 6)	1.1	(1.0, 1.2)	26/91 (29)	(20, 39)

[a] Seronegative indicates ≤1:4 HAI titer. "All" indicates all children, regardless of pre-vaccination serostatus.

[b] 95% CI indicates 95% confidence interval.

[c] GMFR indicates Geometric Mean Fold Rise (Post-Vaccination GMT ÷ Pre-vaccination GMT).

[d] FluMist used in Year One of Study AV006.

6.3.4. Conclusions from Immunogenicity Studies with FluMist in Children

- Immunogenicity of a single dose of FluMist in children increases with increasing dosages, with the highest response rate occurring at dosages of 10^6 to 10^7 $TCID_{50}$.
- A two-dose primary regimen of FluMist was more immunogenic in children than a one-dose primary regimen, particularly for the H1N1 strain.
- FluMist induced heterotypic antibody responses in children against the A/Sydney/05/97 H3N2 drifted strain.
- Consistency lots of FluMist induce similar antibody responses to each of the constituent vaccine strains when administered to children.